

Figure 1

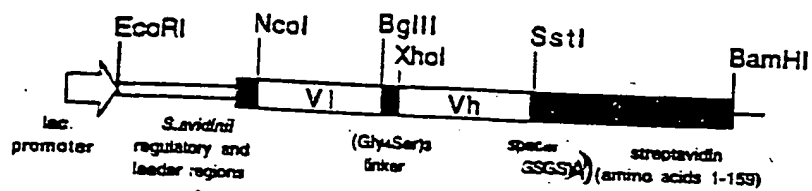


Figure 2

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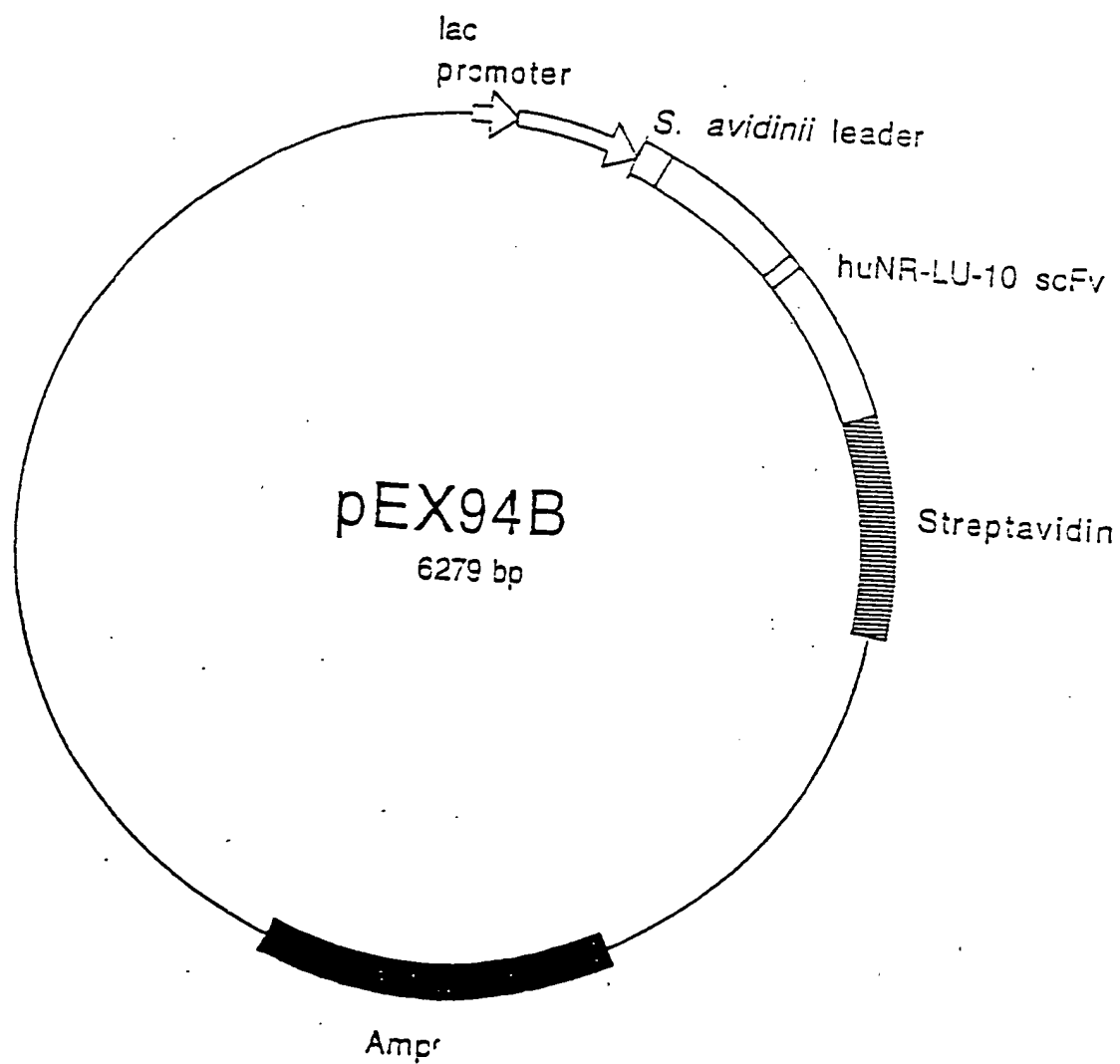


Figure 3

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Signal
Sequence →

```

1      5'  CGCTCCGTCGCGCGCGCGGCAACAACCTAGCGAGTATTTTCGTGTCTCAG
          -20
30  Met Arg Lys Ile Val Val Ala Ala Ile Ala Val Ser Leu Thr Thr
    ATG CGC AAG ATC GTC GTT GCA GCC ATC GCC GTT TCC CTC ACC ACC
    Met
95  Val Ser Ile Thr Ala Ser Ala Ser Ala Asp Pro Ser Lys Asp Ser
    GTC TCG ATT ACC GCC AGC GCT TCG GCA GAC CCC TCC AAG GAC TCG
          10
140 Lys Ala Glu Val Ser Ala Ala Glu Ala Gly Ile Thr Gly Thr Trp
    AGG GCC CAG GTC TCG GCC GCC GAG GCC GCC ATC ACC GGC ACC TGG
          20
185 Tyr Asn Glu Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala Asp
    TAC AAC CAG CTC GGC TCG ACC TTC ATC GTG ACC GCG GGC GGC GAC
          30
230 Gly Ala Leu Thr Gly Thr Tyr Glu Ser Ala Val Gly Asn Ala Glu
    GGC GCC CTC ACC GGA ACC TAC GAG TCG GCC GTC GCG AAC GCC GAG
          40
275 Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser Ala Pro Ala Thr
    AGC CGC TAC CTC CTC ACC GGT GGT TAC GAC AGC GCC GCG GGC ACC
          50
320 Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala Trp Lys Asn
    GAC GGC AGC GGC ACC GCC CTC GGT TGG ACC GTC GCC TCG AAG AAT
          60
365 Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly Glu Tyr
    AAC TAC CGC AAC GCC CAC TCC GCC ACC ACC TGG AGC GGC CAG TAC
          70
410 Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Glu Trp Leu Leu Thr
    GTC GGC GGC GCC GAG GCC AGC ATC AAC ACC CAG TGG CTC CTC ACC
          80
455 Ser Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly
    TCC GCC ACC ACC GAG GCC AAC GCC TGG AAG TCC ACC CTC GTC GGC
          90
500 His Asp Thr Phe Thr Lys Val Lys Pro Ser Ala Ala Ser Ile Asp
    CAC GAC ACC TTC ACC AAG GTG AAG CCG TCC GCC GCC TCC ATC GAC
          100
545 Ala Ala Lys Lys Ala Gly Val Asn Asn Gly Asn Pro Leu Asp Ala
    GCG GCC AAG AAG GCC GCC GTC AAC AAG GCC AAC CCG CTC GAC GCC
          110
          120
          130
          140
          150
          HincII
          Val Glu Glu Stop
590 GTT CAG CAG TAG TCGCGTCCCGGACCGCGCGGTGCGCGGACCTCGGCC 3'

```

Figure 4

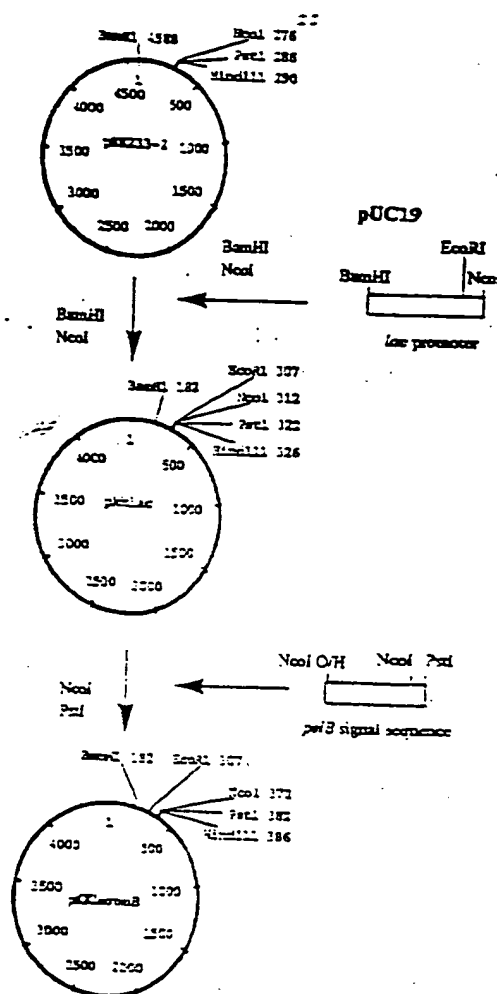


Figure 5

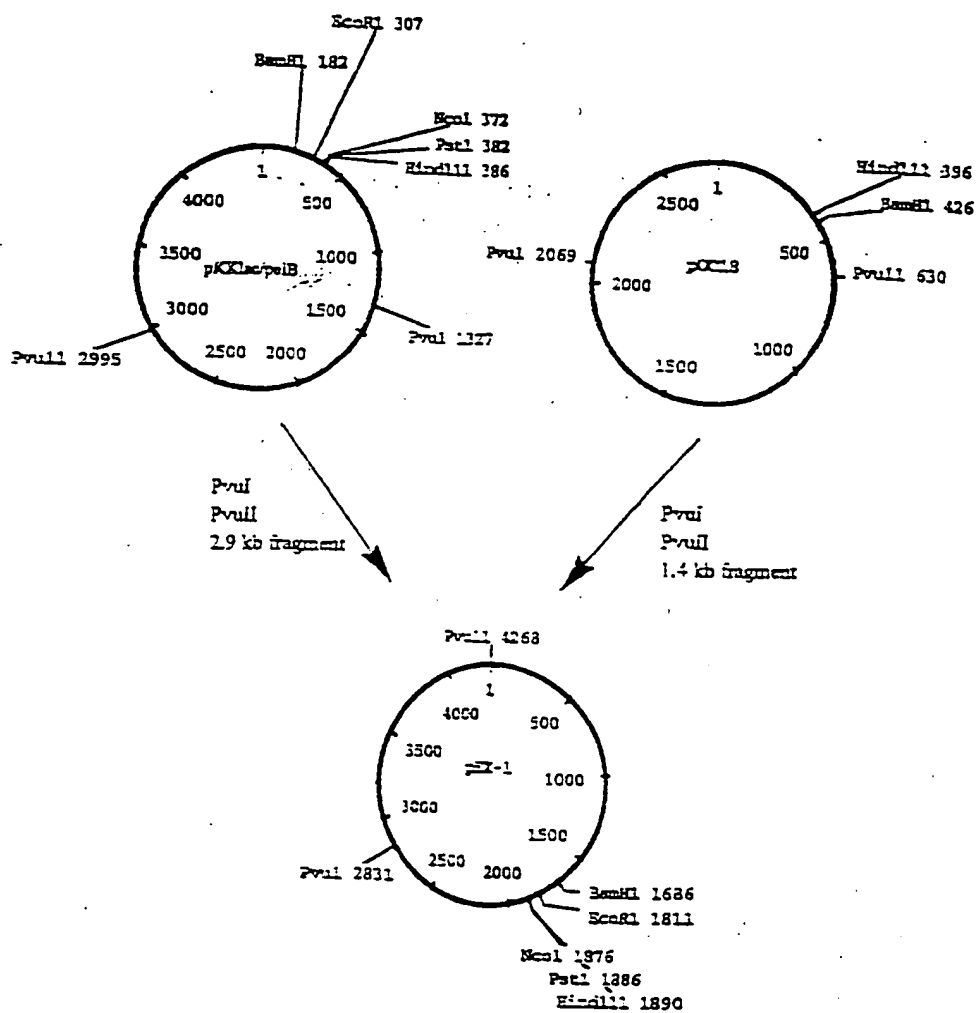


Figure 6

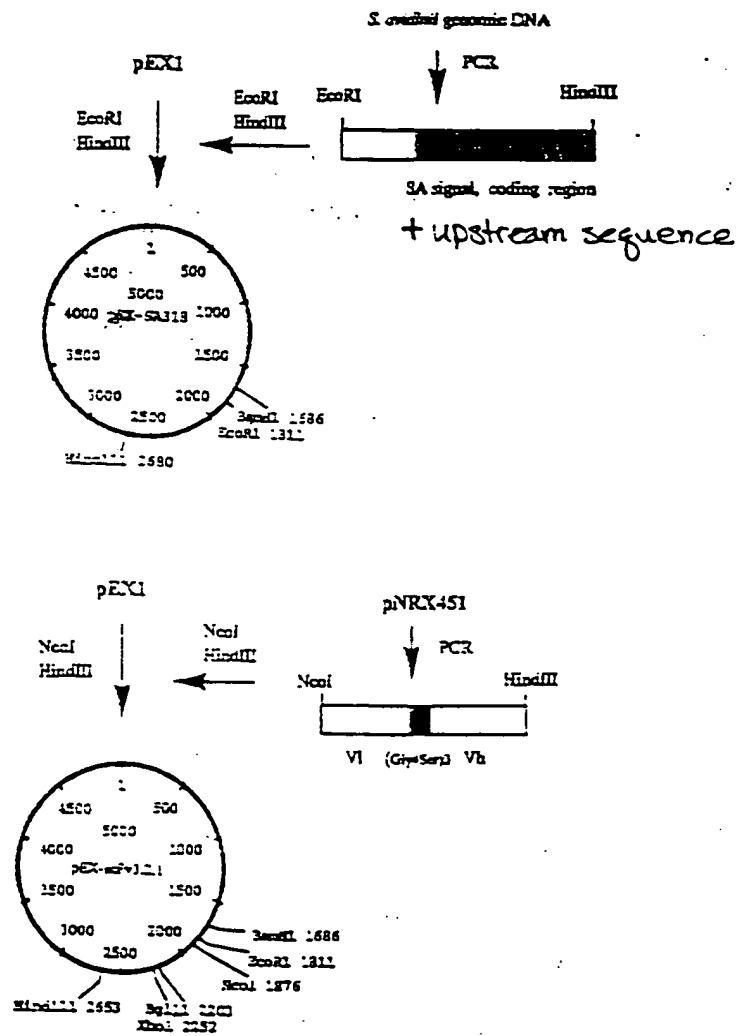


Figure 7

pEX94B

HindIII
ScaI
1.5 kb
fragment

BamHI, fill-in
HindIII

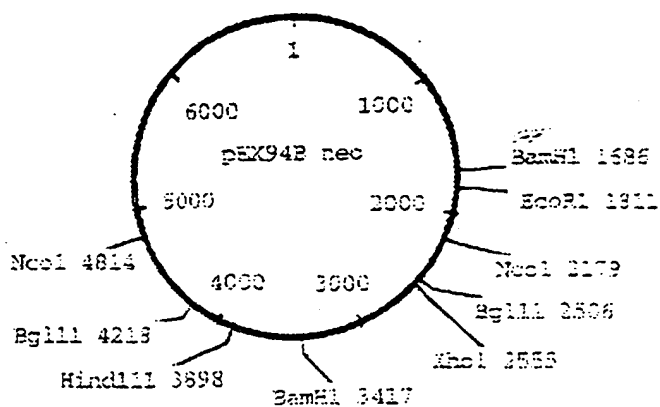
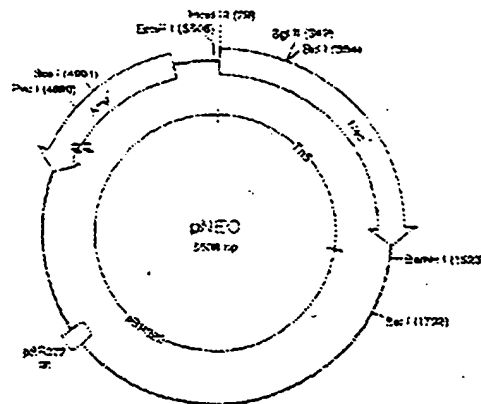


Figure 9

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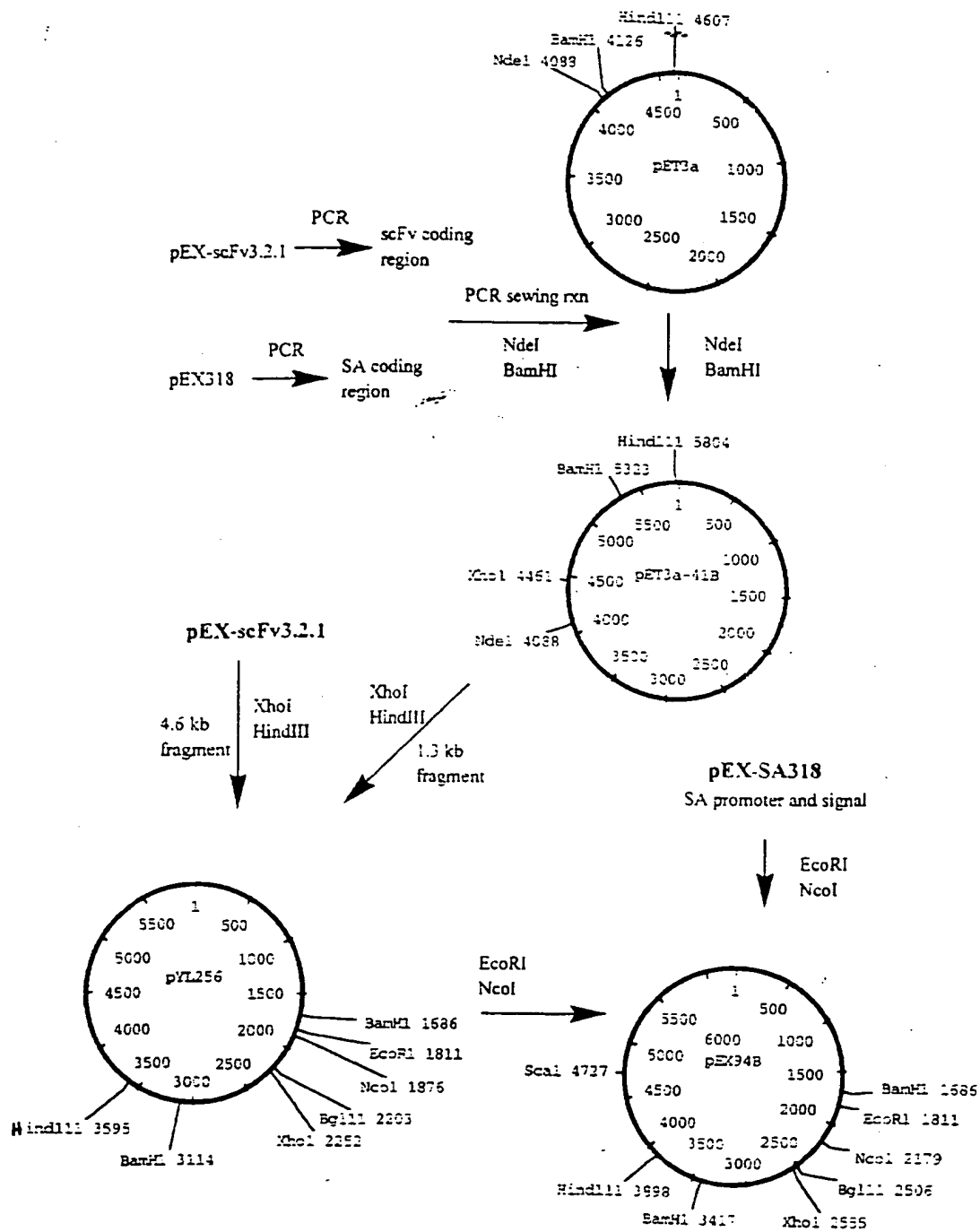


Figure 8

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Figure 10

PEX94B BccRI-BamHI fragment

1 GAATTCACGAGTAAACCGACGGACTCGGCCATTCTTTGGCCGAAATTCCTTTGCAGAAA
61 AATGTTGTTGAGAACCCCTCCGATGCTAGTACGATTACACCAACATGTGCCCTTGGCAA
121 CCATCCACCCCGACCTCGACCATCGAGTTCTGCGCCGAAAGACACATGCCGCACTGCTGT
181 TTGTTACCCGACACCGTCAGGTGCACGGCCGAGGTCAAAACCTGACGGGCGGGATACG
241 GACCGCGCACTCCACAGCGGCGCCCTCGTCCCGCGCGGCAACAACTAGGGAGTATTTTT
301 CGTGTCTCAGATGCGCAAGATCGTCTGTGACGCCATCGCCGTTTCTGACCCAGGTCTC
M R K I V V A A I A V S L T T V S
361 GATACCGGCTATGGCTGACATCCAGACGACTCAGTCTCCATCGTCTTGTCTGCTCTGT
121 I T A M A A D I Q M T Q S P S S L S A S V
signal peptide
421 GGGAGACAGAGTCAACATCACTTGTCTGGGCTAGTCAAGGCATTAGAGGTAATTTAGACTG
141 G D R V T I T C R A S Q G I R G N L D W
481 GTATCAGCGAAACCTGTGAGGACCGGAACTCTTAATCTACTCCACATCCAAATTTAAA
161 Y Q Q K P G K G P K L L I Y S T S N L N
541 TTCTGGTGTCCCATCAAGGTTCACTGCGCACTGGGCTCTGGGTCAATTATCTCTCACCAT
181 S G V F S R F S G S G S G S D Y T L T :
601 CAGCAGCCTTCAAGCTCAAGATTTCCGCACTTAATCTGTCTACAGCCTAATGCGTATCC
201 S S L Q P E D F A T Y Y C L Q R Y A Y P
661 GTACACCTTCGGACAGGGACCAAGCTGGAGATCAAGATCTCTGTTGGCGGTGGGCTCGG
221 Y T F G Q G T K L E I K I S G G G G S G
linker
721 CGGTGGTGGGTCGGGTGGCGAGGCTCAAGCCAGGTTCAAGTCTGCTCAGTCTGGGGCAGA
241 G G G S G G G G S S Q V Q L V Q S G A E
781 GGTGAAAAGCCAGGGGCTCACTCAAGGTGTCTGCAAGGCTTCTGGCTTCAACATTA
261 V K K P G A S V K V S C K A S G P N I K
841 AGACACCTATATGCACCTGGGTGAGGCGAGGCACTGGACAGGGCCTGCACTGGATGGGAAG
281 D T Y M H W V R Q A P G Q G L Q W M G R
901 GATGATCTGCGAATGGTAATACTAAATCCGACCTGTCTCTCAGGGCAGGGTGACTAT
301 I D P A N G N T K S D L S P Q G R V T I
961 AACAGCAGACACCTCCATCAACACAGCCTACATGGACTCAGCAGCCTGAGGTCTGACGA
321 T A D T S I N T A Y M Z L S S L R S D D
1021 CACTGCGCTCTATTACTGTCTAGAGAGTCTCACTGCGACGTTGTTCTTGGACTACTG
341 T A V Y Y C S R E V L T G T W S L D Y W
1081 GGGTCAGGAACCTTAGTCAACCTGAGCTCTGCTCTGGTTCGGCAGCCTTCCAGGA
linker

streptavidin
regulatory
region

streptavidin
signal sequence

V_L

V_H

Figure 10 Cont.

361 G Q G T L V T V S S G S G S A D P S K D
 1141 CTGGAAGGCCCAGGTCTCGGCGCGAGGCGCGATCAGCGGCTCTTGCTACACCGCT
 381 S K A Q V S A A E A G I T G T W Y N Q L
 1201 CGGCTCGACCTTCATCGTGACCGCGGCGCGACCGCGCGCTGACCGGAACCTACGATC
 401 G S T F I V T A G A D G A L T G T Y E S
 1261 GGCGCTCGGCAACGCGCGAGAGCGGCTACGTCCTGACCGGCTGTTACGACAGCGCGCGCGC
 421 A V G N A E S R Y V L T G R Y D S A P A
 1321 CACCGACCGGAGCGCGACCGCGCTCTGCGTTGGACCGGCTGGGCTGGAAGAATACTACCGCA
 441 T D G S G T A L G N T V A W K N N Y R N
 1381 CGCGGCTCTCGGCGACCGACGTTGGAGCGCGCGCTACGTCCTCGCGCGCGCGGCGCGAGGATCAA
 461 A H S A T T N S G Q Y V G G A E A R I N
 1441 CACCGAGTGGCTGCTGACCTCGCGGCGCGACCGAGGCGCGACCGCTGGAAGTCCACGCTGGT
 481 T Q W L L T S G T T E A N A W K S T L V
 1501 CGGCGACGACACCTTCACCGAGGTGAAGCGCTCTCGCGCTCTCATCGACCGCGCGGAAGAA
 501 G H D T F T R V K P S A A S I D A A K K
 1561 GGCGGCGCTCAACAACCGGCAACCGCTCGACCGCTTACGAGTAAGGATCC
 521 A G V N N G N P L D A V Q Q *

streptavidin
1-159

GACATCGTGC TGTGCGCAGTC TCCAGCAATC CTGTCTGCAT CTCAGGGGA GAA
 GGTACAATG CTGTCAGGG CCAGCTCAAG TGTAAGTAC ATCTGGT ACCAGCAGAA
 GCCAGGATCC CCAAAC CTTGGATTGA TCCACATCC AAGGCTT CTGGAGTCCC
 TGCTCGCTTC AGGCGCAGTG GGTCTGGGAC CTCTACTCT CTCAATCA GCAGAGTGA
 GGCTGAAGAT GCTGCCACTT ATTACTGCCA GCAGTGGATT AGTAACCCAC CCACGTTCCG
TGCTGGGACC AAGCTGGAGC TGAAGATCTC TGGCTGGAA GGCAGCCCGG AAGCAGGTCT
GCTCCGGAC GCAGGTTCCG GCTCGAGCCA GGTTCAGCTG GTCCAGTCAG GGGCTGAGCT
 GGTGAAGCCT GGGGCCTCAG TGAAGATGTC CTGCAAGGCT TCTGGCTACA CATTACCAG
 TTACAATATG CACTGGGTAA AGCAGACACC TGGACAGGGC CTGGAATGGA TTGGAGCTAT
 TTATCCAGGA AATGGTGATA CTTCTACAA TCAGAAGTTC AAAGGCAAGG CCACATTGAC
 TGCAGACAAA TCCTCCAGCA CAGCCTACAT GCAGCTCAG AGCCTGACAT CTGAGGACTC
 TGCGGTCTAT TACTGTGCAA GAGCGCAATT ACGACCTAAC TACTGGTACT TCGATGTCTG
 GGGCGCAGGG ACCACGGTCA CCGTGAGCTC TGGCTCTGGT TCGGCA GACC CCTCCAAGGA
 CTCGAAGGCC CAGGTCTCGG CCGCCGAGGC CGGCATCACC GGCACCTGGT ACAACCAGCT
 CGGCTCGACC TTCATCGTGA CCGCGGGCGC CGACGGCGCC CTGACCGGAA CCTACGAGTC
 GCGCGTCGGC AACGCCGAGA GCCGCTACGT CCTGACCGGT CGTTACGACA GCGCCCCGGC
 CACCGACGGC AGCGGCACCG CCTCGGTTG GACGGTGGCC TGAAGAATA ACTACCGCAA
 CGCCCACTCC GCGACCACGT GGAGCGGCCA GTACGTCCGC GCGCCGAGG CGAGGATCAA
 CACCCAGTGG CTGCTGACCT CCGGCACCAC CGAGGCCAAC GCCTGGAAGT CCACGCTGGT
 CGGCCACGAC ACCTTCACCA AGGTGAAGCC GTCCGCGGCC TCCATCGACG CGGCGAAGAA
 GCGCGCGTC AACACGGCA ACCCGCTCGA CGCCGTTTCA CAGTAA

11A

Translation of B9E9pKOD scFvSA

Figure 11B

DIVLSQSPAIL SASPGEKVTM TCRASSSVSY MHWYQKPGS SPKPWYATS NLASGVPARF
 SGSGSGTSYS LTISRVEAED AATFYCQQWI SNPTFGAGT KLELKIS GLE GSPEAGLSPD
AGSGSQVQL VQSGAEL VKP GASVQMSCKA SGYTFTSYNM HWVKQTPGQG LEWIGATYPG
 NGDTSYNQKF K GKATLTADK SSSTA YMQLS SLTSEDSAVY YCARAQLRPN YWYFDVWGAG
 TTVTVS SGS SADPKDSKA QVSAAEAGT GTWYNQLGST FVTAGADGA LTGTYESAVG
 NAESRYVLTG RYDSAPATDG SGIALGWTVA WQNNYRNAHS ATTWSGQYVG GAEARINTQW
 LLTSGTTEAN AWKSTLVGHDTFTKVKPSAA SIDAACKAGV NNGNPLDAVQ Q*

V_L
 pKOD
 V_H
 LINKER 2
 SA

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Figure 11C

E31-2-20 plasmid: NcoI-BamHI fragment containing B9E9 Vh- linker-VL-SA gene

1 CCATGGCTCAGGTTCCAGCTGGTCCAGTCAGGGGCTGAGCTGGTGAAGCCTGGGGCCTCAG
1 M A Q V Q L V Q S G A E L V K P G A S V

61 TGAAGATGTCTTGCAGGCTTCTGGCTACACATTTACCAGTTACAATATGCACTGGGTAA
21 K M S C K A S G Y T F T S Y N M H W V K

121 AGCAGACACCTGCACAGGGCCTGGAATGGATTGGAGCTATTTATCCAGGAAATGGTGATA
41 Q T P Q Q G L E W I G A I Y P G N G D T

181 CTTCTTACAATCAGAGTTCAAGGCAAGGCCACATTGACTGCAGACAAATCCTCCAGCA
61 S Y N Q K F K G K A T L T A D K S S S T

241 CAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAA
01 A Y M Q L S S L T S E D S A V Y Y C A R

301 GAGCGCAATTACGACCTAACTACTGGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCA
101 A Q L R P N Y W Y P D V W G A G T T V T

361 CCGTGAGCAAGATCTCTGGTGGCGGTGGCTCGGGCGGTGGTGGGTGGGTGGCGGGCGCT
121 V S K I S G G G G S G G G S G G G S
linker

421 CGGGTGGTGGTGGGTGGGGCGGGCGGGCTCCAGCGACATCGTGTCTGCGAGTCTCCAG
141 G G G G S G G G G S S D I V L S Q S P A

481 CAATCCTGTCTGCATCTCCAGGGGAGAGGTCACAATGACTTGCAGGGCCAGCTCAAGTG
161 I L S A S P G E K V T M T C R A S S S V

541 TAAGTTACATGCAGCTGGTACCAGCAGAGCCAGGATCCTCCCCCAACCTGGATTATG
181 S Y M H W Y Q Q K P G S S P K P W I Y A

601 CCACATCCAACCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCT
201 T S N L A S G V P A R F S G S G S G T S

661 CTTACTCTCTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGC
221 Y S L T I S R V E A E D A A T Y Y C Q Q

721 AGTGGATTAGTAACCCACCCACGTTCCGTGGTGGGACCAAGCTGGAGCTGAAGAGCTCTG
241 W I S N P P T F G A G T K L E L K S S G

781 GCTCTGGTTCCGCAACCCCTCCAAGGACTCGAAGGCCAGGTCTCGGCCGCGGAGGCCG
261 S G S A D P S K D S K A Q V S A A E A G
linker

841 GCATCACCGGCACCTGGTACAACAGGTCGGGCTCGACCTTCATCGTGACCGCGGGCGCCG
281 I T G T W Y N Q L G S T F I V T A G A D

901 ACGGCGCCCTGACCGGAACCTACAGTGGGCGCTCGCAACGCCGAGAGCCGCTACGTCC
301 G A L T G T Y E S A V G N A E S R Y V L

961 TGACCGGTGGTTACGACAGCGCCCCCGGCAACCGGACCGGCGGCGGCGCCCTCGGTTGGA
321 T G R Y D S A P A T D G S G T A L G W T

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Streptavidin

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1021 CCGTGGCCTGGAAGAATAACTACCGCAACGCCCACTCCGCGACCACGTGGAGCGGCCAGT
 341 V A W K N N Y R N A H S A T T W S G Q Y
 1081 ACGTCGGCCGGCGCGAGGCGAGGATCAACACCCAGTGGCTGCTGACCTCCGGCACCACCG
 361 V G G A E A R I N T Q W L L T S G T T E
 1141 AGGCCAACGCCTGGAAGTCCACGCTGGTCGGCCACGACACCTTCACCAAGGTGAAGCCGT
 381 A N A W K S T L V G H D T F T K V K P S
 1201 CCGCCGCCTCCATCGACGCGGCGAAGAAGGCCGGCGTCAACAACGGCAACCCGCTCGACG
 401 A A S I D A A K K A G V N N G N P L D A
 1261 CCGTTCAGCAGTAAGGATCC
 421 V Q Q + G S

FIG. 11C CONTINUED

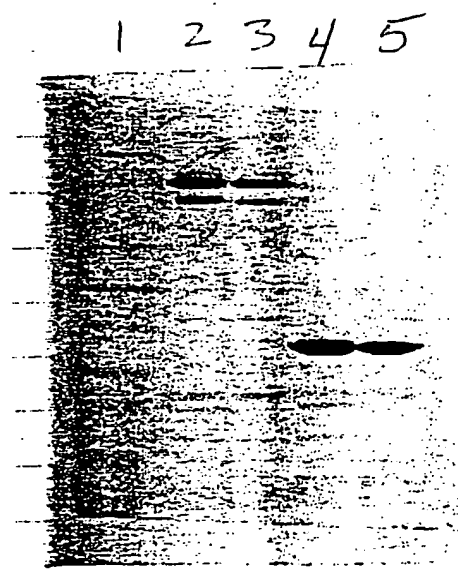


FIG. 12

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Size Exclusion HPLC

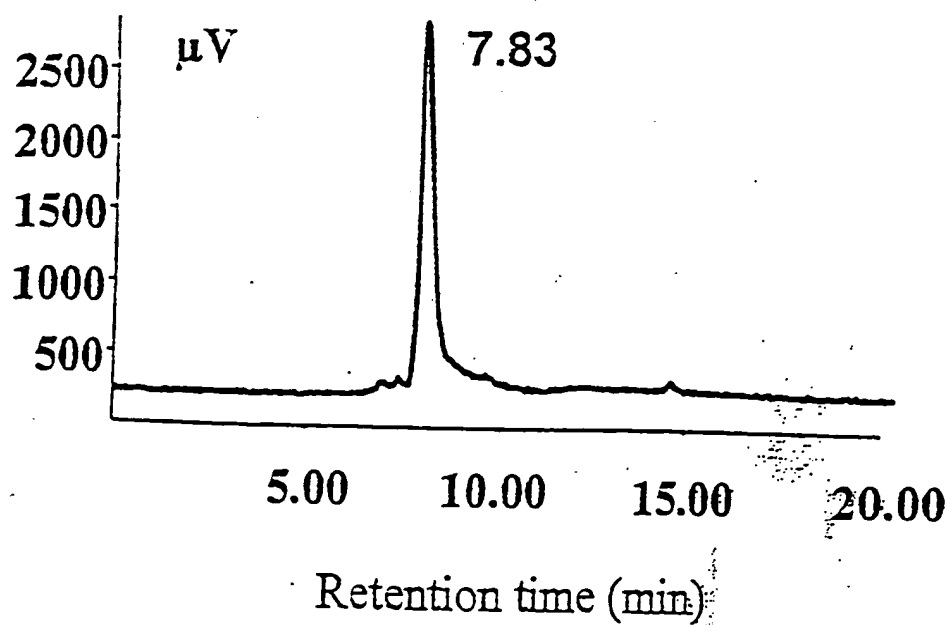


FIG. 13

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Figure 1 is a line graph showing the inhibition of ^{125}I -anti-ScFvSA 97-20.0 binding by ScFvSA 98-01.0 and NrLU10 Mab. The y-axis represents O.D. (Optical Density) ranging from 0.0 to 0.4. The x-axis represents nM ANTIBODY concentration on a logarithmic scale, ranging from 0.1 to 100.0. Three curves are plotted: NrLU10 Mab (squares), scFvSA Standard 97-20.0 (circles), and scFvSA 98-01.0 (diamonds). The scFvSA 98-01.0 curve shows the most potent inhibition, reaching the lowest O.D. values at higher antibody concentrations compared to the other two.

nM ANTIBODY	NrLU10 Mab (O.D.)	scFvSA Standard 97-20.0 (O.D.)	scFvSA 98-01.0 (O.D.)
0.1	0.32	0.29	0.29
0.3	0.30	0.26	0.26
0.6	0.27	0.20	0.20
1.2	0.19	0.15	0.15
2.5	0.17	0.11	0.11
5.0	0.10	0.06	0.06
10.0	0.09	0.04	0.04
20.0	0.05	0.02	0.02
40.0	0.02	0.00	0.00
80.0	0.01	0.00	0.00

226.

224.

FIG. 14

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Dissociation of DOTA-biotin
from scFv-SA at 37 °C 1051-011

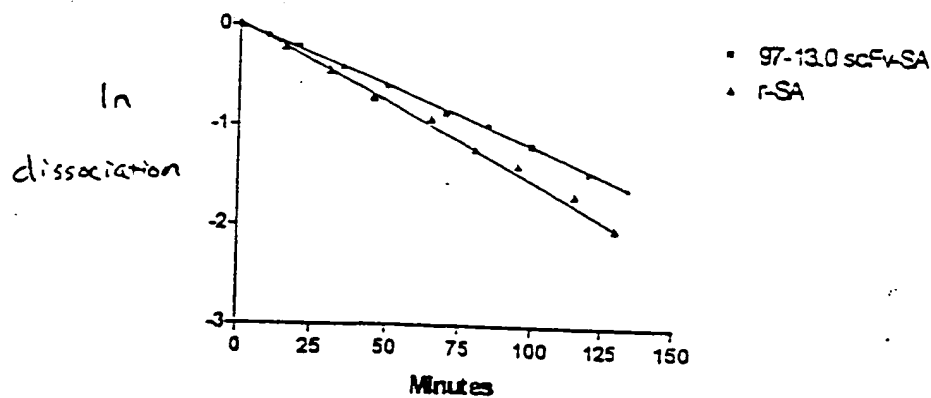


FIG. 15

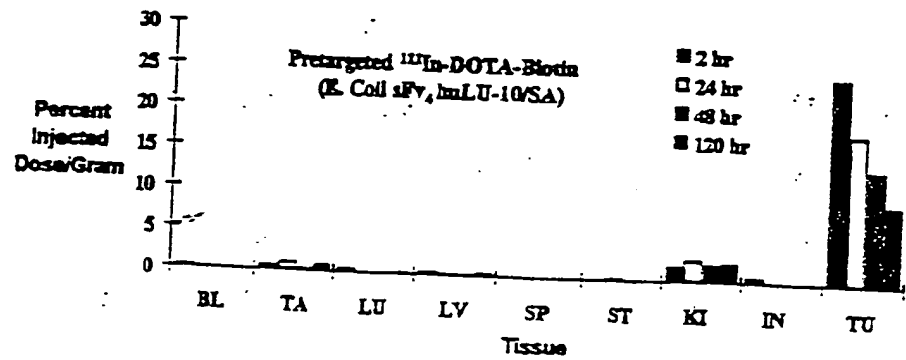


FIG. 16

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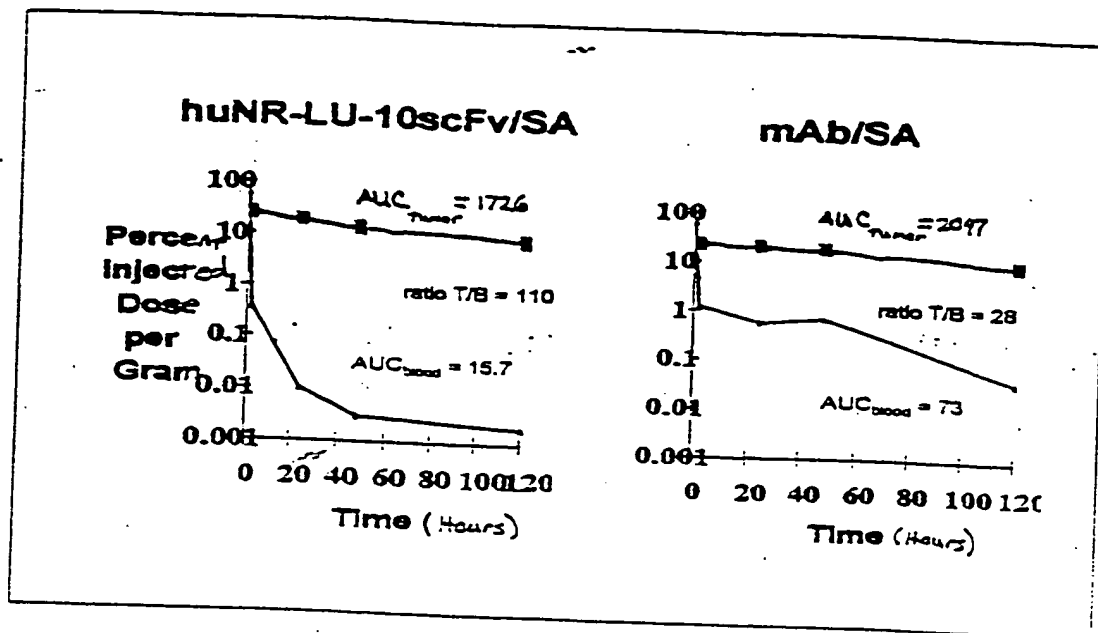


FIG. 17

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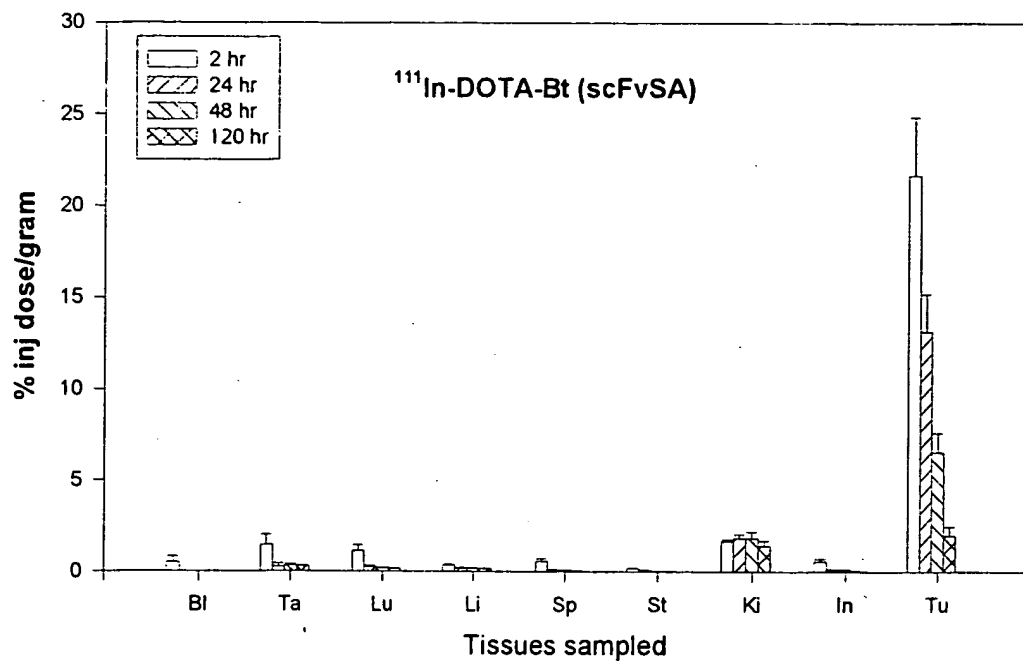


FIG. 18

Expression of scFvSA fusion proteins with or without FkpA.

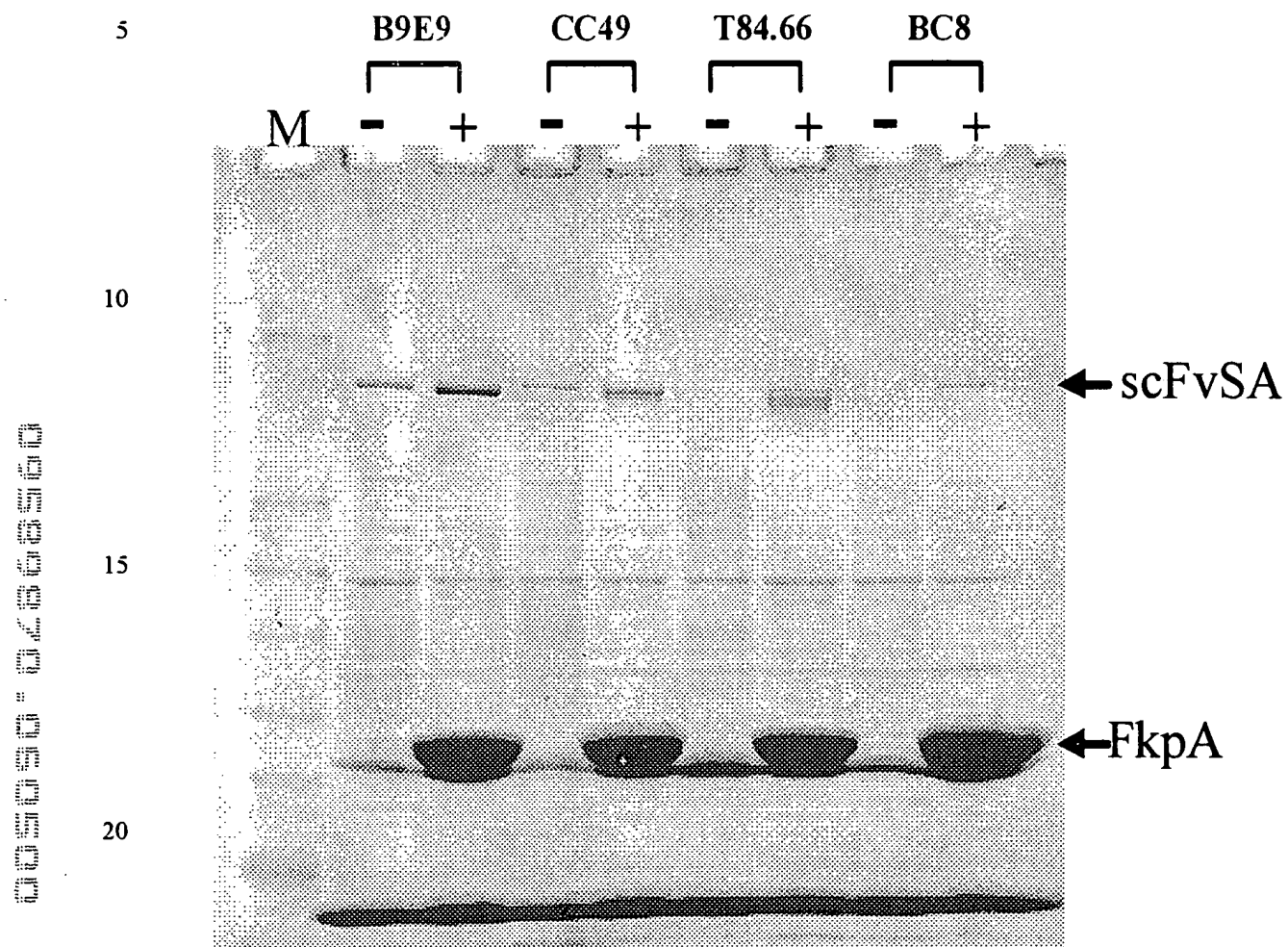


FIG. 19